Impact of Iron Source and Concentration on Rice Flavor Using a Simulated Rice Kernel Micronutrient Delivery System

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ABSTRACT

An extruded grain designed to look like a rice kernel fortified with one of two sources of iron (elemental iron and ferrous sulfate), with and without multiple fortificant (zinc, thiamin, and folic acid), was mixed with milled Calrose rice at low (1:200), medium (1:100), and high (1:50) concentrations. The intensities of water-like, sour taste, hay-like musty, and alfalfa/grassy/green bean flavors were enhanced by the addition of ferrous sulfate ($FeSO_4$) or $FeSO_4$ plus multiple fortificants. Astringent mouthfeel was likewise affected by addition of $FeSO_4$ or $FeSO_4$ plus multiple fortificants. Overall, the elemental iron with multiple fortificants least affected the oxidation of the extruded kernals. Lipid oxidation products in stored fortificant increased the first two to three months and concentrations were higher in samples with $FeSO_4$ as the iron source.

Iron deficiency anemia is one of the most prevalent nutritional problems in the current world population. To combat this deficiency, nutritionists recommend iron fortification of foods. Fortification is more effective than supplementation because nutrients are incorperated into the regular diet, eliminating the problem of easily omitted or forgotten doses (Cook and Reusser 1983). Rice is a dietary staple for a major portion of the world's population and is thus an obvious vehicle for iron fortification (Beck 1971).

Iron availability varies according to the individual and the form of iron ingested. Pregnant women need more iron than menstruating women and both need more than men and menopausal women. Soluble ferrous iron salts (Fe^{+2}) result in greater absorption than ferric salts (Fe^{+3}). Insoluble ferrous salts (ferrous phosphate) have poor absorption, while soluble ferric salts (ferric chloride) have fair absorption. Ferrous sulfate is fairly efficient in treating iron deficiency anemia. Other sources of iron such as elemental or reduced iron are used in the food industry. It dissolves in the hydrochloric acid in the stomach. Therefore, the smaller the particle size, the greater the absorption (Waddell 1974). Both Fe^{+2} and Fe^{+3} forms of iron exhibited discoloration in extruded fortificant products unless acidity was increased before extrusion (Kapanidis and Lee 1996).

Enrichment and fortification of rice can be accomplished in several ways. Currently, most processors of rice use powders and coated grains for enrichment. The powder consists of a preblended mixture of vitamins and minerals that is incorporated with the grains. However, rice rinsing removes the powder. Coated grain-type fortification is an alternative method in which grains of rice are first coated with vitamins and minerals and then coated with a water-insoluble, food-grade material (Hoffpauer 1992).

Another approach for enrichment or fortification is to make simulated grains containing the nutrients. In the late 1980's, an extruded rice fortificant was developed primarily as a method to fortify rice with vitamin A (Lee et al 2000). The product is similar in shape to a rice grain and is added to rice at the 1/50 to 1/200 level. Two challenges with extruded rice fortificants are 1) to make it stable to oxidation and 2) to make it white enough so that it cannot be distinguished when diluted with milled rice (Murphy et al 1992). Iowa State University scientists and the Bon Dente Co. (Lynden, WA) collaborated to increase the stability of the product fortified with Vitamin A (Murphy et al 1992). Early attempts to co-fortify extruded rice fortificant with vitamin A and iron resulted in oxidation of vitamin A by the iron and subsequent discoloration of the product (Murphy 1996).

The objective of this research was to determine how differing sources and amounts of iron fortificant affect the flavor of milled rice, as determined by descriptive analysis. The iron fortificant was an extruded kernel formulated with and without other micronutrients (zinc, folic acid, and thiamin). The combination of these micronutrients may increase oxidation. Therefore, oxidative stability was determined by gas chromatographic analysis of lipid oxidation products.

MATERIALS AND METHODS

Preparation of Fortified Rice

The extruded premix grain-like kernels had five formulations: 1) multiple fortificant product without iron consisting of zinc, thiamin, and folic acid; 2) formula 1 with ferrous sulfate ($FeSO_4$) as the iron source; 3) formula 1 with elemental iron (Fe) as the iron source; 4) $FeSO_4$ alone; and 5) Fe alone. The ratios of premix to rice were 1:50 (high), 1:100 (medium), and 1:200 (low), with 1:100 representing the average target fortification (total of 15 treatment combinations). Each sample was presented twice. The ratios were mixed on a w/w basis. The high concentration mix was prepared with 12 g of premix to 588 g of rice. The medium concentration had 6 g of premix to 594 g of rice. The low concentration had 3 g of premix to 597 g of rice. The levels of nutrients added to this extruded kernel were thiamin (0.45 mg/100 kg), zinc (3.0 mg/100 kg), folic acid (0.15mg/100 kg), and iron (3.8 mg/100 kg). These concentrations are within the normal range of fortification for rice. The distribution in rice could vary from 1:50 to 1:200, depending on the rice consumption and needs of the target population and the micronutrient intake desired.

Sensory Analysis

Twelve panelists previously trained in the principles and concepts of descriptive analysis (Meilgaard et al 1999) participated in the study. The rice flavor lexicon employed was based on the work of Goodwin et al (1996) and Bett-Garber et al (2001) (Table I). It includes 12 unique flavor attributes. Flavor was determined by smelling and by evaluation in the mouth. The average of the most and least intense experience for an attribute in the sample was recorded. Intensity was rated using a 0–15 anchored universal intensity scale (Table II) with 0 indicating not detectable and 15 indicating more intense than most foods (Meilgaard et al 1999). Scores were recorded on a computerized ballot system (DSA-1989, Compusense, Guelph, ON, Canada) Each sample was presented to the panelists twice in separate sessions following the randomized design in which each session consisted of three experimental

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samples, and the standard and blind control (both Calrose commercial rice). The standard was used as a warm-up sample and was presented at the beginning of each session for the panelists to calibrate themselves. Following the warm-up sample, coded test samples were presented to panelists individually at 10-min intervals immediately after cooking (10 min of holding, then portioning into serving cups). Evaluations were conducted at individual test stations under red lights masking color. Distilled, filtered water (Hydrotech drinking water filtration system) and unsalted soda crackers were used to cleanse the mouth between samples.

Cooking Methods

The 600-g portions of rice were rinsed three times in cold water covering the rice, strained to remove excess water, and then transferred to preweighed rice cooker insert bowls. Water was added to give rice-to-water weight ratios of 1:1.5. Following the procedure described by Bett-Garber et al (2001), rice was presoaked in the cooker insert bowl for 30 min at room temperature, cooked in the rice cooker-steamer (Panasonic SR-W10GHP) to completion, held 10 min at the "warm" setting, and sampled. Cooking of samples were staggered and analyzed by the panel at 10-min intervals.

Statistical Analysis

Data were analyzed with SAS software using PROC MIXED. The experimental design was a split-plot design. The main unit has an incomplete block design with the main unit treatment (fixed effects) being five types of fortificants plus a control (Calrose rice). The blocking factor was the panel session effect. The session and panelists were random effects. The LSMEANS statement was used to determine significant differences between the various fortificants, the three concentrations, and all of the combinations thereof. The denominator degrees of freedom (DDFM) are calculated with the Kenward Roger option (kr) in the model statement.

Gas Chromatography Methods

Four formulations were analyzed by gas chromatography to determine the development of a representative lipid oxidation product (nonanal) during storage: 1) Fe; 2) Fe plus multiple fortificant (zinc, folic acid, and thiamin); 3) $FeSO_4$; and 4) $FeSO_4$ plus multiple fortificant. The storage conditions were ambient (25°C) and accelerated (40°C). Samples were stored in cotton bags and zip-closure polyethylene bags and sampled at 0, 1, 2, 3, 4, 5, and 6 months.

Samples for analysis consisted of 0.75 g of rice in 2-mL vials with Teflon seals. Samples were spiked with a 2-µL aliquot of a solution of 1 ppm of 2,4,6-trimethylpyridine (TMP) in water, which served as the internal standard. Samples were preheated for 5 min at 60°C before sampling. Collection of volatile compounds was accomplished using a 15-min adsorption period at 60°C while agitating the sample. The solid phase microextraction (SPME) fiber employed was a 1-cm 50/30 carboxen/DVB/PDMS fiber (Supelco). Collected volatiles were desorbed for 1 min on a GC/MS system (HP5973, Agilent). The injector temperature was held constant at 270°C. The GC oven temperature was held for 1 min at 50°C, then ramped to 250°C at 10°C/min. Volatile compounds were separated on a DB-5 capillary column (30 m × 1.0 m, i.d.) with helium as the carrier gas under a constant flow of 40 cm/sec. The mass spectrometer was operated in scan mode from m/z 50 to m/z 350 using electronic ionization. Peak areas were determined for each compound by integrating a target ion unique to that compound. Each treatment was analyzed in triplicate, integrated peak areas were averaged, and conclusions were drawn from visual observations.

RESULTS

Water-like/metallic flavor of rice arises from the aromatics and mouthfeel of the minerals and metals commonly associated with tap water (Goodwin et al 1996). The effects of iron fortification on this flavor in rice were dependent on iron source and concentration and possibly the addition of zinc oxide (Fig. 1A). Water-like/ metallic flavor was significantly more intense in rice fortified with $FeSO_4$ plus multiple fortificant at high and medium concentration than in the control. Rice fortified with high level of $FeSO_4$ was more intense than the control but not significantly higher. Fortification of rice with $FeSO_4$ at lower concentrations, with and without the multiple fortificant, did not significantly affect water-like/ metallic flavor in the rice. Addition of multiple fortificant without iron resulted in water-like/metallic flavor intensities similar to those resulting from the addition of $FeSO_4$ plus multiple fortificant at higher concentrations of 0.078 µg and 0.038 µg/100 g of rice, but were not significantly different than the control. It appears that the zinc (oxide) in the multiple fortificant, like the $FeSO_4$, enhances the water-like/metallic flavor. Whether these intensity changes are detectable to a target consumer population remains to be determined.

TABLE I
Descriptive Sensory Analysis Attributes and Definitions Used to Evaluate Cooked Rice Flavor

Sewer/Animal An immediate and distinct pungent aromatic in the flavor characterized as sulfur-like and generic animal. Animal aromatic in the flavor can sometimes be identified as "piggy".

Floral Aromatics associated with dried flowers such as lilac or lavender. This aromatic is characterized as spicy floral as in an old fashioned sachet.

Grain/Starchy A general term used to describe the aromatics in the flavor associated with grains such as corn, oats and wheat. It is an overall grainy impression characterized as sweet, brown, sometimes dusty, and sometimes generic nutty or starchy.

Hay-like/Musty A dry, dusty, slightly brown aroma/flavor with a possible trace of musty.

Popcorn A dry, dusty, slightly toasted and slightly sweet aromatic in the flavor that can be specifically identified as popcorn.

Corn Sweet aromatics of the combination of corn kernels, corn milk, and corn germ found in canned yellow creamed-style corn.

Alfalfa/Grassy/Green Beans A dried, green, slightly earthy, slightly sweet aroma/flavor including grassy and fresh green bean aroma/flavor.

Dairy A general term associated with the aromatics of pasteurized cow's milk. Most apparent just before swallowing.

Sweet Aromatic A sweet impression such as cotton candy, caramel, or sweet fruity that may appear in the aroma and or aromatics.

Water-like/Metallic Aromatics and mouthfeel of the minerals and metals commonly associated with tap water. This excludes any chlorine aromatics that may be perceived.

Sweet Taste Basic sweet taste associated with sugar.

Sour/Silage A sour fermented vegetation aroma/flavor, not decaying vegetation.

Astringent Chemical feeling factor on the tongue, described as puckering or dry and associated with tannins or alum.

TABLE II
Universal Flavor Scale and References

Descriptor	Reference	Intensity
Sweet	1% sucrose solution	1
Oil	Frito Lay potato chips	2
Diacetyl	Land-O-Lakes margarine	3
Grape	Kool Aid (grape)	4
Apple	Mott's Natural apple sauce	5
		6
Orange	Minute Maid orange juice	7
		13
		14
Grape	Welch's grape juice	10
		11
Cinnamon	Wrigley's Big Red gum	12
		13
		14
		15

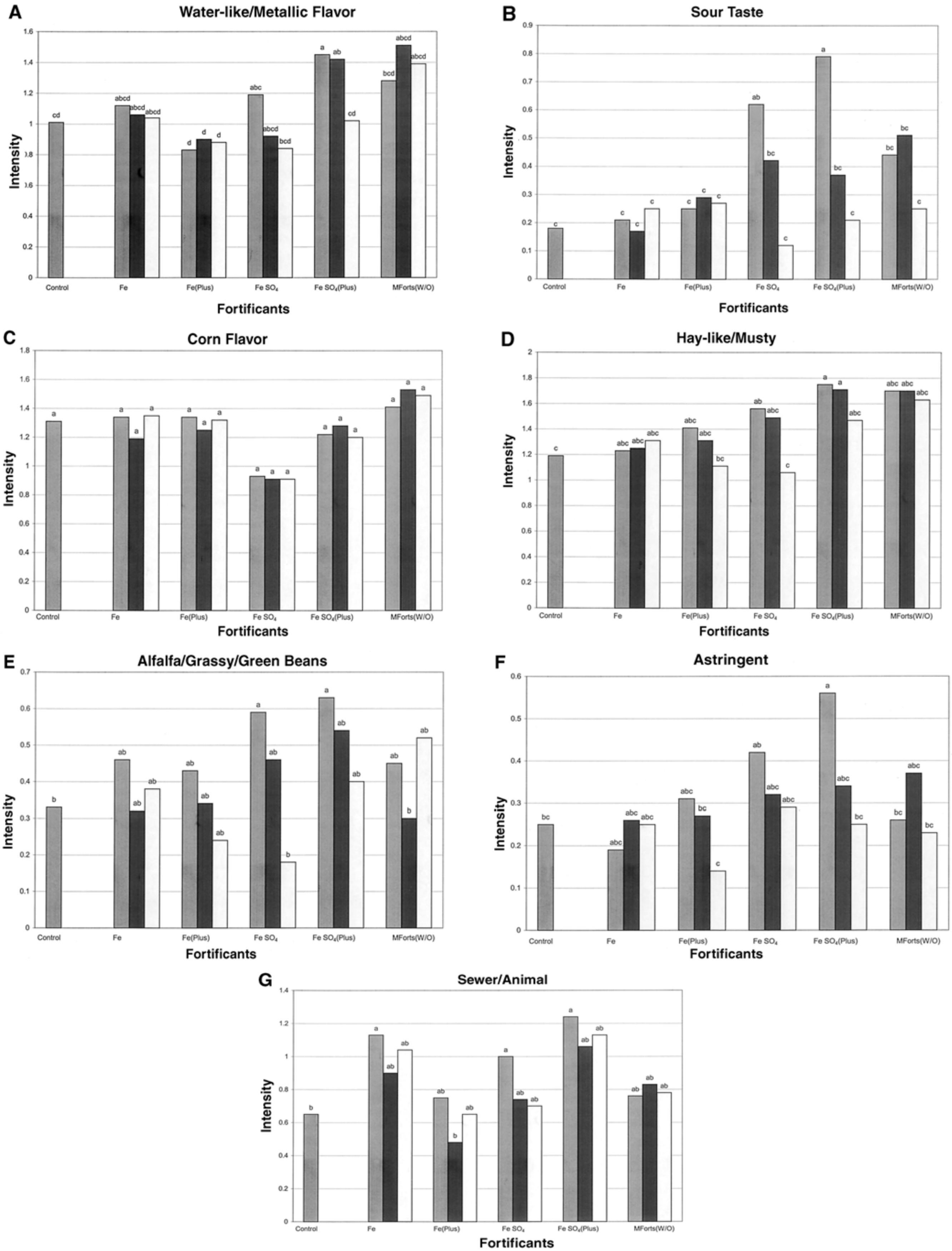

Fig. 1. Sensory attribute means of treatment combinations (fortificant treatment by ratio). Fe, elemental iron; Fe(Plus), elemental iron plus multiple fortificant; $FeSO_4$, ferrous sulfate; $FeSO_4$(Plus), ferrous sulfate plus multiple fortificant; Mforts(W/O), multiple fortificant without iron. **A,** water-like/metallic flavor; **B,** sour taste; **C,** corn flavor; **D,** hay-like/musty; **E,** alfalfa/grassy/green beans; **F,** astringent; **G,** sewer/animal. Bars with the same letter have means that are not significantly different based on LSMEANS analysis.

The addition of elemental iron (Fe) alone or in combination with the multiple fortificant did not (statistically) significantly affect water-like/metallic flavor compared with the control. Addition of Fe caused a more intense water-like/metallic flavor than Fe plus multiple fortificant, although it was not significant. Why the synergistic effect between Fe and the multifortificant reduced water-like/metallic flavor to consistently less than iron alone, multifortificant alone, or the control is not understood.

Sour taste was significantly higher in rice fortified with $FeSO_4$ or $FeSO_4$ plus multiple fortificant at the high concentration than in the control (Fig. 1B). Sour taste was also significantly higher at the higher concentration of these fortificants than at the low concentration. Addition of the multiple fortificant without iron resulted in an increase in sour taste over the control at the high and medium concentrations, although not significantly different. Zinc oxide in the multiple fortificant, like $FeSO_4$, appears to enhance sour taste. Sour taste at the high concentration of multiple fortificant was less intense than that of rice fortified with $FeSO_4$ or $FeSO_4$ plus multiple fortificant in this mix, although it was not significant. Although at very low concentrations, sour taste was less intense in the rice fortified with a low concentration of $FeSO_4$ than in the control, but not statistically significant. The addition of Fe with and without other fortificants resulted in sour taste similar in intensity to that of the control. As observed for water-like/metallic flavor, the Fe suppressed the effect of the multiple fortificants on sour taste. The intensities of sour taste were very low from an overall flavor standpoint. The intensity at which sour taste becomes objectionable is not known but it is probably higher than the intensities seen here, because these intensities are in the average range for rices (Bett-Garber et al 2001).

Grain/starchy is a natural flavor component in rice, and the effect of its increase or decrease on consumer acceptance is unknown. Addition of iron with or without multiple fortificant did not significantly affect it (data not shown). The trend for corn flavor appeared to be suppressed by addition of $FeSO_4$ at all ratios, but the changes were not statistically significant (Fig. 1C). If corn flavor is perceived as a negative sulfury corn note, suppression of the flavor may have a positive impact on consumers. No other fortificants significantly affected corn flavor, and no trends were observed. Sweet taste was not significantly affected by fortificants (data not shown).

The hay-like flavor includes a musty characteristic. The high and medium concentrations of all fortificants, except Fe, increased the intensity of this attribute (Fig. 1D). The $FeSO_4$ plus multiple fortificants and multiple fortificants without iron increased hay-like flavor at all three concentrations. These intensities are about average for rice (Bett-Garber et al 2001). Therefore, as long as there is not a dominant musty note, the majority of consumers would not reject the fortified rice based on these small changes in intensity.

Alfalfa flavor, which includes a green grassy flavor, increased significantly in intensity with higher concentrations of $FeSO_4$ and $FeSO_4$ with multiple fortificants (Fig. 1E). Overall, the intensities are low (<1 point) and may not affect consumer acceptance.

Astringent mouthfeel was increased by high concentrations of $FeSO_4$ and $FeSO_4$ plus multiple fortificants (Fig. 1F). At low intensities (<0.6), it is doubtful that this would be a problem. Calrose, the rice used in this work, is generally low in astringent intensity (Bett-Garber et al 2001). In rice with a more intense innate level of astringency, any enhancement could be perceptible to consumers, especially if it doubles the intensity as the high concentration of $FeSO_4$ did with multiple fortificants.

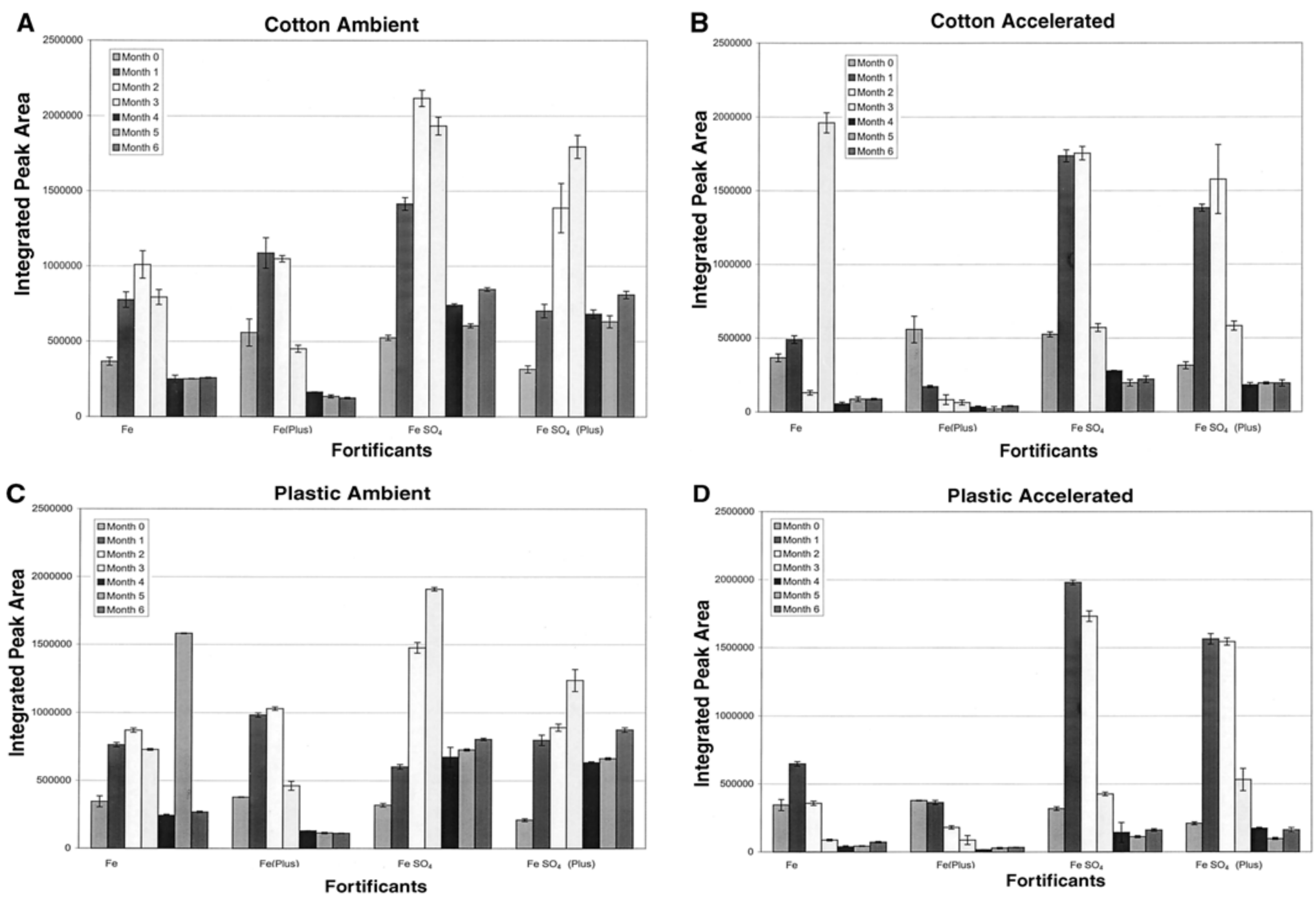

Fig. 2. Gas chromatography mean values of integrator count for treatment combination (fortificant by storage time). Fe, elemental iron; Fe(Plus), elemental iron plus multiple fortificant; $FeSO_4$, ferrous sulfate; $FeSO_4$(Plus), ferrous sulfate plus multiple fortificant; Mforts(W/O), multiple fortificant without iron. **A,** 25°C in cotton bag; **B,** 40°C in cotton bag; **C,** 25°C in a sealed plastic bag; **D,** 40°C in a sealed plastic bag.

Sewer/animal flavor is an off-flavor in rice and the cause is unknown. Fe and $FeSO_4$ plus multiple fortificant at all three concentrations seem to cause the greatest increase in this characteristic (Fig. 1G), although only the high concentration was significantly different from the control. $FeSO_4$ at the high concentration also caused a significant increase.

Milled rice is whiter than all four of the fortificants used in this experiment. $FeSO_4$ plus multiple fortificants was the darkest of the formulations. Elemental iron plus multiple fortificants was whiter than elemental iron. Fortification with these products could be obvious to the consumer. There is concern that consumers would separate out the fortified grains and discard them because of the color difference.

Shelf Life Analysis

The graphs in Fig. 2A–D depict changes in the concentration of nonanal, a lipid oxidation product, during storage. Similar graphs can be generated for other lipid oxidation products such as hexanal, 1-pentanol, hexanoic acid, 2-heptanone, or combinations thereof, which gave similar results. Although trimethyl pyridine was added to all samples as an internal standard, a large variation in its recovery was observed, rendering any normalization techniques suspect. The recovery of the internal standard was noticeably less in samples containing $FeSO_4$, which coincidentally contained large concentrations of lipid oxidation compounds in the headspace.

The treatments with multiple fortificants produced less volatile lipid oxidation products than those without. The concentration of volatile compounds increased until month 2 or 3, then a noticeable drop was observed, with only slight variations observed in the concentrations for months 4, 5, and 6. In general, the addition of Fe resulted in a lower concentration of lipid oxidation products in the headspace than $FeSO_4$ by nearly a factor of 2 for storage times of two months and longer. This trend held for both samples with iron plus multiple fortificant and iron only. One outlier was the accelerated elemental Fe in cotton bags at month 3. Relative standard deviation for the triplicate analysis is 3.4%, indicative of great confidence in the measurement. Yet this value is much larger than would be expected without good explanation.

Concentrations were similar for months 1 to 2 when comparing ambient versus accelerated temperature. At extended storage periods, there were more volatile compounds found in rice held at ambient temperature than at accelerated temperature. This was true regardless of packages. The expectation was that storage at accelerated temperature would promote volatile compound formation. A possible explanation is that volatile compounds in the heated samples were readily released from the rice before gas chromatography analysis.

Packaging appears to have made little difference, especially over an extended period of time. The accelerated treatments gave similar results for plastic and cotton packaging at months 4, 5, and 6. The same held true for the ambient stored rice over all four treatments.

CONCLUSIONS

Descriptive analysis demonstrated the flavor changes that occurred in the base rice due to the fortificant. Overall, Fe with multiple fortificant affected the flavor of the base rice the least. The intensities of water-like, sour taste, hay-like musty, and alfalfa/grassy/green bean flavors were enhanced by the addition of $FeSO_4$, $FeSO_4$ plus multiple forticant, and multiple forticant without iron at the high concentrations. Astringent mouthfeel was likewise affected by addition of $FeSO_4$ and $FeSO_4$ plus multiple fortificant. $FeSO_4$ addition at the low concentration (0.019 μg/100 g of rice) appeared to suppress the intensities of the flavor attributes (water-like, sour taste, corn, hay-like/musty, alfalfa/grassy/green bean) relative to the control rice.

These descriptive analyses results give no indication of consumer preference. Although the changes in flavor intensities with iron and multiple fortificant additions were small, the effects on consumer acceptance remain to be determined. The information from these descriptive flavor analyses can assist with practical formulation decisions (i.e., source of iron) before taking the product to the consumer. It can also help with interpreting and understanding consumer data. For example, if consumers disliked the $FeSO_4$ with fortificant, then it could be assumed that an increase in water-like/metallic compounded with a decrease of some characteristic rice flavors would affect consumer acceptance.

Rice fortified with Fe alone or with multiple fortificant had better storage characteristics than that fortified with $FeSO_4$ alone or with multiple fortificant, as indicated by relative changes in nonanal, a lipid oxidation product.

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